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A Pilot Multi-Center International Double-Blind Placebo Controlled Randomized Study of Sulindac, a Pan-COX Inhibitor, in Oral Premalignant Lesions

THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Introduction

Oral precancerous lesions (OPL) represent a valuable model for clinical trials for tobacco related cancers. However, due to the relatively low prevalence of this condition in the United States, subject accrual to such trials is slow. Conversely, in India, the prevalence of oral leukoplakia is among the highest in the world. Indeed oral cancer, caused by exposure to tobacco smoke, alcohol and betel nut quid, is the leading cause of cancer deaths in India.

To date, there are no effective treatments documented in randomized controlled clinical trials to prevent malignant transformation of leukoplakia. However, evidence that non-steroidal anti-inflammatory drugs (NSAIDs) prevent experimental and animal head and neck cancer, and colon and breast cancer in humans lends support to the promise of NSAIDs in the chemoprevention of oral cancer.

The purpose of this protocol is to pilot a multi-center chemoprevention trial of sulindac, a pan-cyclooxygenase (COX) inhibitor, for oral leukoplakia through an international collaboration between Memorial Sloan-Kettering Cancer Center (MSKCC), New York and the Amrita Institute of Medical Sciences (AIMS) and the Regional Cancer Centre (RCC), in Cochin and Trivandrum, India. Specifically, we will conduct a 66 subject, 2-arm, double-blind, placebo-controlled randomized study of sulindac 150 mg bid to test the clinical efficacy, safety and molecular effects of sulindac against OPL and OPL tissue. Oral leukoplakia subjects will be enrolled from AIMS, RCC and MSKCC, however, we expect that most subjects will be recruited from AIMS and RCC due to the substantially higher prevalence of this condition among the Indian compared to the US population.

MSKCC will be the coordinating center for this trial, and will thus be responsible for all aspects of clinical trial design and management. Our study team, in collaboration with the Office of Clinical Research and the Office of the Physician-in-Chief, has spent a considerable amount of time and effort in developing a comprehensive data and safety monitoring (DSM) plan. **An informal, yet critical objective of this trial is to determine the feasibility of conducting this multi-center trial and to further refine the operating procedures and DSM plan for future studies.**

Objectives

Primary Objective

To evaluate the efficacy of sulindac in subjects with early or advanced oral premalignant lesion (OPL) by both clinical response (reduction in size of all lesions) and histological response (change in histological grade).

Secondary Objectives

To evaluate the effect of sulindac in modulating the expression of the intermediate biomarkers Ki67, p53 proteins and DNA ploidy after 24 weeks of treatment of study drug.

To evaluate the correlation between baseline COX-2 expression or DNA ploidy with clinical response or biomarker modulation

To evaluate the safety of chronic dosing of sulindac in this subject population

To explore the relationship between genetic polymorphisms of genes involved in carcinogenesis and clinical or biomarker response to sulindac

DESIGN

Subjects will have a histologically suspected or confirmed index oral premalignant lesion, 12mm or greater in size that has not been biopsied in the past 6 weeks. Subjects will be stratified by clinic site, and an index oral premalignant lesion will be identified and stratified as either:

EARLY:
presence of at least atypical cells; or mild dysplasia; or hyperplastic leukoplakia of high-risk sites, lateral and ventral tongue, and floor of mouth

or

ADVANCED:
presence of moderate dysplasia; and/or
presence of severe dysplasia (excluding CIS).

Sixty-six subjects will be randomized either to placebo or sulindac 150 mg bid treatment stratified by clinic site and early or advanced lesions.

While receiving study drug, subjects will have clinic visits every 4 weeks. Subjects will have the index lesion (or lesion area) biopsied and histologically confirmed at registration, and after 24 weeks of treatment. The oral evaluations performed at 24 weeks will be used to determine final response.

Biopsies and blood samples will be used for analysis of biomarkers. These include: COX-2 mRNA (at baseline), DNA ploidy, p53 protein, Ki67 protein, and genetic polymorphisms. IHC for p53 and Ki67 are to be assessed at AIMS and MSKCC; samples from RCC will be sent to AIMS for analysis. Methodology for DNA ploidy status is currently under development. COX-2 levels will be measured in Dr. Dannenberg's laboratory, at Weill Medical College of

Cornell University, and future genetic polymorphic analyses will be performed at MSKCC Molecular Epidemiology Laboratory.

The efficacy of sulindac will be evaluated by the proportion of subjects that present with a CR or PR at 24 weeks. The study is powered to provide an 80% chance of seeing a 30% response rate.

Adverse signs and symptoms and concurrent medications will be recorded at 4-week intervals while receiving study drug and at the end of the study. Safety will be assessed by comparing physical exams, clinical laboratory tests and adverse events.

2.1 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objective

- To evaluate the efficacy of sulindac in subjects with early or advanced oral premalignant lesion (OPL) by both clinical response (reduction in size of all lesions) and histological response (change in histological grade)

Secondary Objectives

- To evaluate the effect of sulindac in modulating the expression of the intermediate biomarkers Ki67, p53 proteins and DNA ploidy after 24 weeks of treatment of study drug
- To evaluate the correlation between baseline COX-2 expression or DNA ploidy with clinical response or biomarker modulation
- To evaluate the safety of chronic dosing of sulindac in this subject population
- To explore the relationship between genetic polymorphisms of genes involved in carcinogenesis and clinical or biomarker response to sulindac

3.0 BACKGROUND AND RATIONALE

Epidemiology of Head and Neck Cancer

Head and neck cancers accounted for 3% of all new cancer cases and 2% of cancer deaths in the United States in 1999 (1). Despite multimodality treatment efforts including surgery, radiation therapy, and chemotherapy, the overall survival rates for head and neck cancer subjects have improved only modestly. (2). The main reasons for treatment failure are the development of second primary tumors in subjects with early stage disease (Stage I and II) and the development of local recurrence and metastasis for subjects with locally advanced disease (Stage III and IV) head and neck squamous cell carcinoma (HNSCC) (2).

In the Indian population to be studied, head and neck cancer accounts for 50% of all cancers (3). A large proportion of the population has precancerous oral leukoplakia due to the prevalent habits of tobacco smoking in the form of 'bidi' cigarettes, reverse smoking (lighted end in the mouth), alcohol consumption, and chronic use of betel quid (areca nut, lime, and tobacco) held in the buccogingival sulcus for extended periods. The 'oral precancerous lesion clinic' at AIMS, run by Dr. Kuriakose, sees 40 patients bi-monthly, while Dr. Sebastian and his surgical oncology group at RCC see close to 20-25 subjects who have oral premalignant lesions. The primary risk factors include smoking tobacco and alcohol consumption.

HNSCC results from a multistep carcinogenesis process which occurs over large areas of the upper aerodigestive tract epithelium exposed to carcinogens. This condemned mucosa contains multiple transformed clones that can develop into new primary tumors at a rate of 30% over five years. This process is called "field cancerization" (4). These patients harbor multifocal, metachronous, premalignant and lesions. Clinical efficacy in treating oral precancerous lesions may suggest efficacy of agents in preventing primary or second primary tobacco related cancers.

Oral Premalignant Lesions (OPLs)
Oral premalignant lesions (OPLs) are white and/or red mucosal patches in the upper aerodigestive tract. The histologic appearance of oral premalignancy varies from hyperplasia with atypical cells to severe dysplasia.

The standard of care for OPLs is observation or removal. If the area is extensively involved, or multiplicity prohibits excision, the only alternative is close observation. The recurrence rate after excision of leukoplakia is 35%. Repeated surgical excisions can be associated with scarring and poor functional outcome.

Prospective studies of subjects with OPLs revealed a significant incidence of malignant transformation to SCC depending primarily upon the presence of dysplasia. In the largest U.S. series consisting of 257 untreated oral leukoplakia subjects, Silverman et al., determined the malignant transformation rate at 8 years was 17.5%, however, the rate rose to 36.4% for those with dysplasia (5). None of the dysplastic lesions improved spontaneously. A large study by Silverman of Indian workers with OPL showed similar findings (6). Pindborg reports hyperplastic lesions of high risk sites have increased risk of progression to cancer (99).

Clinical trials of drug therapy for OPLs
Despite many clinical trials with retinoids, the narrow therapeutic window of these agents does not allow their safe routine use for these lesions (7). A randomized, placebo-controlled, double-blind trial evaluated the efficacy of 13-cis-retinoic acid in halting or reversing the development of OPLs (8). A total of 46 subjects were randomized to treatment with 13-cRA (1-2 mg/kg/day) or placebo for three months, with six further months of follow-up. Intolerable conjunctivitis and hypertriglyceridemia developed in 2 subjects receiving 2 mg/kg. Among the 24 13-cRA subjects, 2 had complete responses, 14 had partial responses, however relapse occurred 2-3 months after 13-cRA therapy ended.

Another randomized, double-blind trial was designed to evaluate low-dose 13-cRA versus β-carotene in maintaining remission of oral premalignancy following induction therapy with high-dose 13-cRA (9). At the conclusion of induction, the rate of response was 55% (36 subjects), and the rate of stable disease was 35% (30 subjects). Of the 59 subjects included in the second phase, 53 were evaluable. Of these, 22 in the 13-cRA group and 13 in the β-carotene group responded to maintenance therapy or continued to have stable lesions (92% vs. 45%).

The biochemoprevention study employing a combination of 13-cRA, alpha-tocopherol and alpha-interferon was designed to address advanced premalignant lesions of the upper aerodigestive tract that are resistant to single agent retinoid intervention. At 6 months, 31 subjects were evaluable for response: 12 had a pathologic complete response, 7 partial response, and at 12 months, 8 complete and 7 partial responses (10).

Celecoxib (marketed under the brand name of Celebrex™) is a FDA approved drug for treatment of the signs and symptoms of osteoarthritis and rheumatoid arthritis, and for the reduction in the number of adenomatous colorectal polyps in FAP. Celebrex was tested for efficacy in the management of oral leukoplakia in a recently completed trial at MSKCC and MDACC (results pending). That study was initiated because of the known dramatic over expression of Cyclooxygenase –2 (COX-2) in head and neck cancers and leukoplakia compared to true normal tissue, and the known high levels of prostaglandins that may contribute to carcinogenesis in these subjects. Cyclooxygenase exists in two forms: a constitutive form (COX-1) and an inducible form (COX-2). COX-1 is normally produced by a wide variety of cells throughout the body, including the stomach, platelets and kidney. COX-2 is associated with inflammation and pain and is increased in expression at inflammatory sites and in many cancers and precancers. Celecoxib is a specific inhibitor of the inducible form of the cyclooxygenase enzyme. Inhibition of COX-1 and COX-2 is an important strategy for cancer treatment and prevention trials in many organ systems. NSAIDS including sulindac, that inhibit COX activity are widely effective in animal models of cancer prevention including head and neck cancer.

Sulindac

Sulindac trademarked Clinoril by Merck, is an NSAID approved for arthritis but known to prevent colon polyps in humans and widely studied for cancer prevention through the NCI. Sulindac is a prodrug that is metabolized to two active moieties, which competitively inhibit COX-1 and COX –2 enzymes. It has been proven effective as a chemopreventive in several animal models of chemically induced cancer (11-13). It has been tested in humans for the prevention of colon cancer (14). The principal toxicities are the potential for upper GI ulceration, and platelet dysfunction, and renal toxicity.

Rationale for NSAIDs in chemoprevention

Current chemoprevention strategies in several areas are focusing on developing agents that inhibit the activity of COX enzymes. One effect of these cancer prevention mechanisms is down-regulation of prostaglandin synthesis in premalignant and malignant tissue. Another potential mechanism by which COX contributes to carcinogenesis is by catalyzing not only the synthesis of prostaglandins but by activating the metabolism of carcinogens to reactive mutagenic electrophiles (15, 16). Increased levels of prostaglandins have been detected in multiple epithelial cancers, including those of head and neck origin (17).

Prostaglandins are important in the post-initiation phases of tumorigenesis as they modulate immune surveillance by inhibiting the immune response to malignant cells, affecting cell proliferation and promoting angiogenesis (18, 19). Both COX-1 and COX-2, encoded by separate genes, catalyze the synthesis of prostaglandins. COX-1 is a constitutive enzyme with constant levels of expression and COX-2 is an inducible isoform encoded by an early response gene that is induced by growth factors, tumor promoters, oncogenes and carcinogens (20-23). Overexpression of COX-2 in epithelial cells inhibits apoptosis, which increases the tumorigenic potential of initiated cells (18).

COX catalyzes the oxidation of B[a]P-7,8-dihydrodiol to B[a]P-diolepoxide, which is a highly reactive and a strongly mutagenic carcinogen (24). The COX enzyme system is especially important in bioactivating chemicals in extrahepatic tissues, such as head and neck, where the P-450 mono-oxygenase system has low activity. COX converts a broad array of carcinogens to reactive metabolites which can form DNA adducts. Thus, several mutagenic derivatives are activated by COX. By analogy to the inducing effects of B[a]P on selected P-450s, benzo[a] pyrene upregulates COX-2 gene expression in human normal and transformed oral epithelial cells through increased transcription, linking COX-2 to the pathogenesis of smoking-related cancers, including head and neck cancer (22).

Upregulated COX-2 expression was found in HNSCC (25). Mean levels of COX-2 mRNA were increased by 150-fold in HNSCC compared with normal oral mucosa from healthy volunteers, and a 50-fold increase in COX-2 mRNA in normal-appearing epithelium adjacent to HNSCC. IHC shows induction of COX-2 in oral leukoplakia.

Sulindac has not been previously studied as a chemopreventive agent for HNSCC. A definitive trial of the chemopreventive efficacy of sulindac for HNSCC would require a large and lengthy randomized trial using an endpoint like cancer onset as the primary outcome. Such a study requires some knowledge of a clinically relevant effect in the population of interest as well as preliminary evidence that sulindac may have chemopreventive qualities and is worth studying further in this population. This pilot study is designed to explore if sulindac is worth carrying forward into a larger study and to provide the information necessary to design such a study. In addition, an informal objective of this study is to determine the feasibility of conducting a multi-center international clinical chemoprevention study among subjects in the US and in the high risk Indian population. By randomizing subjects, we can also observe whether this subject population will agree to participate in this type of study and

be randomized to treatment or placebo. This information is vital for assessing whether future studies can be carried out in collaboration with AIMS and/or RCC.

Rationale for selection of Sulindac:
As outlined above, there is extensive clinical and preclinical data suggesting a role for COX-2 in carcinogenesis and for COX inhibitors for treatment and prevention of cancers. Selective COX-2 inhibitors are being extensively studied in this regard. However, in recent months, two reports have suggested new evidence of cardiotoxicity for these agents (94, 95) leading to renewed interest in pan COX inhibitors such as sulindac due to a long history of safety in the post marketing setting. Sulindac has been proven to reduce precancerous lesions in the human colon and so represents an ideal agent to test in tobacco related cancer prevention (14). Recently one preclinical study shows sulindac causes apoptosis in head and neck cancer cell lines (96) and another reports efficacy of sulindac for inhibiting precancerous markers in a mouse model of lung carcinogenesis (97, 98). Taken together with the continued interest in sulindac by the NCI, we selected sulindac as the best agent to use in this international trial. Sulindac represents the best balance of safety and efficacy.

Rationale for the Proposed Study Population
Oral precancers represent a valuable model for clinical trials for tobacco related cancers, but in the United States, accrual to these trials is slow due to the relatively low incidence of dysplastic oral leukoplakia in the United States. An example of accrual difficulties is a current multi-institutional trial of celebrex in oral precancer that has accrued just 43 subjects (half the goal) in 2 years accrual time at MSKCC, MDACC, and three other institutions. Because large randomized trials of highly promising agents are prohibitively lengthy even at the largest cancer centers, it is logical to look to more endemic areas of this disease, such as Kerala India, in which to perform these important clinical trials.

Dr. Kuriakose, a US trained Head and Neck Surgeon and adjunct Professor at NYU Medical Center, has returned to Kerala, India to lead the Head and Neck Surgery Service at a modern well equipped Cancer Center, the Amrita Institute for Medical Sciences (AIMS). Dr. Paul Sebastian, the Head of Surgical Oncology and Chief of the Head and Neck Service at RCC in Trivandrum, India has over 20 years of clinical experience and great expertise and interest in prevention and early detection of oral cancers. Dr. Sebastian has been at MSKCC as an observing surgeon from March of 2008 to May of 2008. (please see Appendix VII. Description of AIMS and RCC).

This provides an opportunity to establish an international clinical chemoprevention collaboration in this high-risk area.

The primary objective of this pilot multi-center international randomized clinical trial is to determine clinical efficacy of sulindac (provided by NCI) for oral leukoplakia. Indeed, this 66 subject study is powered for an 80% chance of identifying a 30% difference in response rates between the treatment and control arms. However, an informal yet critical objective of this trial is to evaluate the important feasibility questions of performing such international multi-

center trials. This trial will demonstrate that at AIMS and RCC, compliant subjects can be accrued and retained in adequate numbers, that they can be adequately screened, informed, and monitored for safety, and that the clinical data and specimens and biomarker data can be reliably gathered for analysis. With the participation and oversight of MSKCC, the trial will be conducted at all centers in compliance with NIH guidelines, standards for the protection of human subjects in clinical research, and the privacy rule.

The agent sulindac has a well-known favorable safety profile helping to insure the safety of participants. The incidence of significant GI toxicity is low and assessable by frequent monitoring of symptoms, which is feasible in this trial setting. Because sulindac is a COX inhibitor, this trial builds on current hypotheses that these agents may be efficacious in tobacco related cancers and so these data are likely to advance the field.

Clinical trials show an increased risk of cardiovascular events when COX-2 selective and nonselective NSAIDs are used long-term. There is no data indicating specific or significant cardiovascular risk when using sulindac and the overall safety profile of sulindac is excellent.

Biomarker Rationale

Tumorigenesis of the aerodigestive tract is thought to represent a multistep process leading to an increased risk of carcinogenesis (26-28). The carcinogen-induced damage is thought to accumulate with time of exposure and drive the tissue through a series of changes, which involve genetic alterations, dysregulation of proliferation, differentiation, and cell death, as well as an increased capability to invade surrounding tissue.

Studies have been performed to characterize the genetic changes and their phenotypic consequences in tissues undergoing the tumorigenesis process, or in tumors which have resulted from this process. As a result, a variety of markers in the tumorigenesis process have been developed that can be utilized during chemoprevention trials. These markers may assess both risk of tumor development in a tissue at risk, as well as the effect of the chemopreventive intervention on the premalignant tissue.

The capability to ascertain tumor risk and to measure tissue response can add significant power to clinical chemoprevention trials for several reasons. First, the level of tumor risk development from premalignant lesions, while higher than that in tissues without premalignant lesions, is still low enough to require large numbers of participants. The characterization of markers that can identify those individuals at highest risk will serve to make chemoprevention trials more efficient by including only those subjects who might derive benefit from the trial. In addition, it could reduce the number of participants who are subjected to the potential side effects of the intervention and would be unlikely to derive benefit.

The use of biomarkers gives a second source of power to a chemoprevention study by providing surrogate intermediate markers. In most cases, the development of cancer is a long-term process. Therefore, the determination of efficacy in a chemoprevention trial may require many years of follow-up. This not only slows the progress of chemoprevention studies, but it

also limits the number of potential effective agents that can be studied. Once surrogate intermediate markers can be identified and validated, they can be used as short-term indicators of response. This would decrease the length of time necessary to evaluate responsiveness to intervention. In addition, the use of surrogate intermediate markers will help to characterize the pathobiology of response and thus provide insights into new strategies for chemopreventive intervention. In many cases, it is not fully understood why particular chemopreventive agents are effective in reversing or halting the tumorigenesis process. The development of biomarkers will help achieve a better understanding of how the agents work at the tissue level. This will then lead to new rationales for treatment.

Based on this rationale the proposed trial will also incorporate the study of candidate intermediate biomarkers of the head and neck tumorigenesis process. As secondary endpoints, the modulation of several genotypic and phenotypic candidate biomarkers for the head and neck tumorigenesis process are targeted in an effort to define aspects of the tumorigenesis pathway. The biomarker candidate studies proposed are: 1) COX-2 mRNA expression, 2) p53 expression, 3) Proliferation status by Ki67 staining 4) DNA ploidy, a marker of genomic instability that is correlated with risk to progression 5) genetic polymorphisms of genes related to carcinogenesis and sulindac effects.

COX-2 Expression
Levels of COX-2 are increased in HNSCC as well as in normal-appearing mucosa adjacent to HNSCC (25), suggesting that upregulation of COX-2 expression can occur early during the head and neck tumorigenesis process and that down-regulation of its expression may accompany response to chemopreventive intervention. It will be important to correlate baseline levels of COX-2 with clinical response to COX inhibitor therapy with sulindac.

To analyze the expression of COX-2, a sensitive competitive RT-PCR assay has been developed in which the amount of COX-2 mRNA will be measured from small quantities of RNA. This method relies on the co-amplification in the same tube of known amounts of competitor DNA with COX-2 cDNA obtained after reverse transcription from total tissue RNA. The competitor and target use the same PCR primers but yield amplicons with a different size allowing their separation on a gel at the end of the reaction.

p53 Expression
Mutation of the p53 gene has been found in 40-50% of head and neck cancers and is frequently associated with an increased level of p53 product in the cells. It has been found that p53 overexpression is a frequent occurrence in oral premalignant lesions and was associated with decreased response to retinoid chemoprevention and increased rates of progressive disease or cancer (29, 30). Although p53 overexpression in premalignant lesions is not always associated with p53 gene mutation, studies suggest that it might precede gene mutation. In relation to COX, the recent observation that wt p53 suppresses transcription of COX, lends support to the hypothesis that cells that carry alterations in the p53 pathway (which occur frequently in dysplastic oropharyngeal lesions) could express higher levels of COX-2 and thus provide better targets for COX inhibition compared to normal cells. P53

protein expression by immunohistochemistry is a well characterized assay frequently used in many types of clinical cancer therapeutic and prevention trials.

Ki67
Proliferative dysregulation has been a long standing identifiable characteristic of the tumorigenesis process and has been extensively assessed in prior chemoprevention studies. The proliferative fraction of cells in premalignant lesions appears to increase as tissue passes from normal histology to hyperplasia to dysplasia then to cancer (31). Interventions that reduce proliferation are likely to be clinically helpful. Ki67 protein expression measured by immunohistochemistry is a well characterized surrogate endpoint used frequently in chemoprevention trials.

DNA Ploidy
DNA ploidy is a gross marker of genetic abnormalities and has been specifically correlated with the risk of progression of OPL to invasive cancer. As aneuploid lesions represent the highest risk lesions, the efficacy of sulindac in aneuploid lesions is an important assessment. It has been shown in previous work that aneuploidy correlates with elevated COX-2 expression in oral lesions suggesting that NSAID approaches may be efficacious in these lesions. Obviously, if any intervention could normalize aneuploidy this would represent an important finding.

Genetic polymorphisms:
In addition to the above biomarkers, we propose to extract DNA from whole blood for future exploratory genetic analyses. Beyond the scope of this proposal, future investigations will examine whether polymorphisms in candidate genes that code for xenobiotic metabolizing enzymes in carcinogen activation and detoxification pathways (CYP1A1, GSTM1 and GSTMT1) modify the effect of sulindac on final response and biomarker modulation in oral premalignant lesions.

The identification of both genetic and environmental factors that determine an individual's susceptibility for the development of cancer is an important ongoing goal of molecular epidemiology. Most pertinent to the current proposal, susceptibility to oral cancer may be mediated by genetically determined differences in the activity of drug metabolizing enzymes involved in the detoxification and activation of carcinogens derived from tobacco smoke, smokeless tobacco and betel quid constituents. Thus, it is biologically plausible that within the current trial, an individual's response to sulindac treatment may be modified by their ability to metabolically process past or ongoing carcinogenic exposures. Consequently, nested within this randomized clinical chemoprevention trial, we propose to evaluate the effect of sulindac treatment on OPL among subgroups of individuals characterized by candidate genotypes associated with higher cancer susceptibility. The following section will provide the rationale behind this exploratory investigation by briefly describing the main factors of carcinogenic exposure, the predominant enzymes that metabolize such compounds, the prevalence of known polymorphisms of genes that encode these enzymes, and the association of these polymorphisms with cancer risk.

The function of the most intensely studied members of the CYP and GST families, the prevalence and functionality of their known genetic polymorphisms, as well as their association with cancer risk in populations of various ethnicity's is described.

Cytochrome p450's

The CYP family catalyze the oxidative metabolism of most endogenous chemicals (e.g. hormones and fatty acids) and exogenous chemicals (e.g. polycyclic aromatic hydrocarbons, aromatic amines and mycotoxins) (32). CYP1A1, the most intensely studied member of this family, is induced by cigarette-smoke in the oral cavity of humans and by aryl hydrocarbons in vitro epithelial cells (33) and is thought to be responsible for the reactive oxygenated metabolite-mediated oxidative stress of cigarette-smoke constituents, and nitrosamines. Phenotyping studies have shown that the CYP1A1 exon 7 isoleucine/valine substitution polymorphism is associated with relevant functional differences. Importantly, the polymorphic amino acid change from ile/val at amino acid residue 462 of the CYP1A1 enzyme has been shown to result in higher catalytic and mutagenic activity towards benzo[a]pyrene (34), 7-ethoxyresorfin (35-37) and both (R)-6- and 8-hydroxy-warfarin (37, 38).

Linkage between this polymorphism and increased lung cancer risk has been suggested in a recent pooled analysis of 11 international studies including a total of 1950 cases and 2617 controls (39, 40), in Chinese (41), Japanese (42), Brazilian (43), Finnish (44) but not in Swedish (45) populations. Although relatively few studies have reported the frequency of the CYP1A1 polymorphism and its association with oral cancer risk, the first case-control study of the CYP1A1 polymorphism and oral cancer risk showed a prevalence of CYP1A1 exon 7 (ile:val + val/val) genotype in white American male and female oral cancer subjects and controls to be 18% and 8%, respectively, resulting in a statistically significant odds ratio of 2.5 (95% CI=1.2-5.7) (46). Subsequent studies conducted in Japan and Taiwan also found that individuals with the CYP1A1 exon 7 polymorphism were at increased risk for oral cancer and oral precancerous lesions (47, 48). A positive association between the CYP1A1 exon 7 polymorphism and increased oral cancer risk has also been observed in the Indian population (Table 1). Table 1 also shows the high prevalence of the CYP1A1 exon 7 polymorphism in two studies of unrelated healthy individuals (49, 50).

Table 1. Frequency distribution of CYP1A1 genotype in the Indian population

Reference	Disease	No. of cases	No. of controls	Ile/Val genotype among cases (%)	Ile/Val genotype among controls (%)	OR (95% CI)
(32)	Oral cancer	98	60	CYP1A1*2 (51)	CYP1A1*2 (17)	5.3 (1.0-26.3)
(49)	Unrelated healthy individuals	-	139	-	CYP1A1*2 (18)	
(50)	Unrelated healthy individuals	-	883	-	CYP1A1*2 (21)	-

GST's

The GST's are involved in the detoxification of a wide variety of active metabolites of tobacco carcinogens (51, 52). Specifically, GST's are dimeric proteins that catalyze conjugation reactions between glutathione and diverse electrophilic compounds such as aromatic heterocyclic radicals and epoxides in tobacco smoke to produce a less reactive, water-soluble metabolite more readily excreted from the urine. GST enzymes are coded for at five distinct loci, known as alpha, mu, theta, pi and gamma. Two loci in particular, GSTM1 and GSTT1 may be of relevance for susceptibility to HNSCC.

The **GSTM1** locus has been mapped on chromosome 1p13.3, and has been observed to be expressed in the squamous mucosa of the head and neck (53). Because persons with homozygous deletions of the GSTM1 locus have no enzymatic functional activity of the GSTM1 enzyme (54), these individuals potentially accumulate more DNA adducts through their inefficiency at excreting 7,8-diol-9,10-expoxide, the activated carcinogen of the polycyclic aromatic hydrocarbon benzo-[a]-pyrene in tobacco smoke (55).

A recent review of case-control studies (55) indicates that the prevalence of gene variants for GSTM1 varies by ethnicity. Among hospital-based case-control studies conducted in the United States, reported frequencies of the GSTM1 deletion genotype range from 23-41% for persons of African descent, 32-53% for persons of Asian descent, 40-53% for those of Hispanic descent and 35-62% for those of European descent. Population based studies have reported prevalences of 48-57% for the GSTM1 deletion genotype among US Caucasians, while Pacific Islanders and Malaysians have reported GSTM1 deletion genotype frequencies of 62-100%. Other Asian populations, such as the Japanese and Chinese, also have a high frequency of GSTM1 deletions (48-50% and 35-63%, respectively).

Homozygous deletion of GSTM1 has been associated with higher risk of laryngeal, lung (56-64), bladder (65-67), breast (68-70)(35, 36), colon and gastrointestinal cancers (71-73)(12, 37-47) as well as shorter survival among lung cancer patients (74). The epidemiological evidence of an association between the deletion of GSTM1 and oral cancer susceptibility is inconsistent. While several studies suggest the deletion of GSTM1 predisposes an individual to oral cancer (75-80) others do not (46, 81-86). However, studies conducted in Japan consistently indicate that the GSTM1 deletion genotype is associated with increased oral cancer risk (OR range from 1.7-2.2) (87-89). Positive associations between GSTM1 deletion genotype and increased oral cancer risk have also been observed in the Indian population (Table 2a).

Table 2a. Frequency distribution of GSTM1 genotypes in the Indian population

Reference	Disease	No. of cases	No. of controls	Deletion genotype among cases (%)	Deletion genotype among controls (%)	OR (95% CI)
(90)	Oral cancer	297	450	GSTM1 (49)	GSTM1 (24)	3.2 (2.4-4.3)
(32)	Oral cancer	98	60	GSTM1 (49)	GSTM1 (33)	1.34 (0.4-4.8)
(91)	Oral leukoplakia	98	82	GSTM1 (82)	GSTM1 (17)	22 (10-47)
(49)	Healthy individuals	-	139	-	GSTM1 (33)	
(50)	Unrelated healthy individual	-	883	-	GSTM1 (27)	-

Table 2a also shows the high prevalence of the GSTM1 deletion genotype in two studies of unrelated healthy individuals (49, 50).

The **GSTT1** locus has been mapped on chromosome 22q11.2. As with homozygous deletions of the GSTM1 locus, individuals with homozygous deletions of GSTT1 locus have no enzymatic functional activity (54) against its substrates such as epoxybutanes and ethylene oxide derived from tobacco (92).

Studies of GSTT1 null genotype in the United States demonstrate that deletion of GSTT1 is less common than the GSTM1 deletion genotype (55). Among those of European ancestry, 15-31% shows no functional GSTT1 enzyme; African Americans have frequencies ranging from 22-29%; while those of Hispanic origin carry GSTT1 deletions of 10-12%. Asians have

the highest reported GSTT1 deletion genotype. Studies report that 46-58% of Chinese, 38% of Malaysians, and 42-46% of Koreans have the GSTT1 null genotype. While several case-control studies showed an increased risk of oral cancer with the GSTT1 null genotype (OR ranges from 1.5-2.0) (85, 93), others have not (78, 81-83, 88).

Table 2b displays the few studies reporting the frequency of GSTT1 null genotypes among the Indian population.

Table 2b. Frequency distribution of GSTT1 genotypes in the Indian population

Reference	Disease	No. of cases	No. of controls	Deletion genotype among cases (%)	Deletion genotype among controls (%)	OR (95% CI)
(90)	Oral cancer	297	450	GSTT1 (18)	GSTT1 (12)	1.6 (1.04-2.6)
(32)	Oral cancer	98	60	GSTT1 (18)	GSTT1 (8)	2.5 (0.3-21.7)
(91)	Oral leukoplakia	98	82	GSTT1 (76)	GSTT1 (22)	11 (5-22)
(50)	Unrelated healthy individuals	-	883	-	GSTT1 (13)	-

By archiving DNA extractions for future exploratory genetic analyses, we will have the opportunity to advance our understanding of the genetic factors that predispose this heavily exposed population to oral cancer. In addition, such information will allow us to identify commonalities and differences in oral carcinogenesis in this sample of Indian subjects relative to those studied in the US. If the prevalence of such genetic polymorphisms is found to be similar to that in the US, it may be feasible to extrapolate results obtained from the US studies, to that of the Indian population. Conversely, if the prevalence of such genetic polymorphisms is substantially different from that in the US Caucasian population, tailored strategies based on this knowledge will be feasible.

Note: The molecular epidemiologic component will not be completed within the time frame of the proposed study. Indeed, it is likely that the emerging molecular epidemiologic literature on oral cancer and precancer may stimulate further hypotheses we have not currently identified. Thus, upon completion of the study, we will re-evaluate and finalize our selection of candidate pathways and genes that will be investigated in such exploratory genetic analyses.

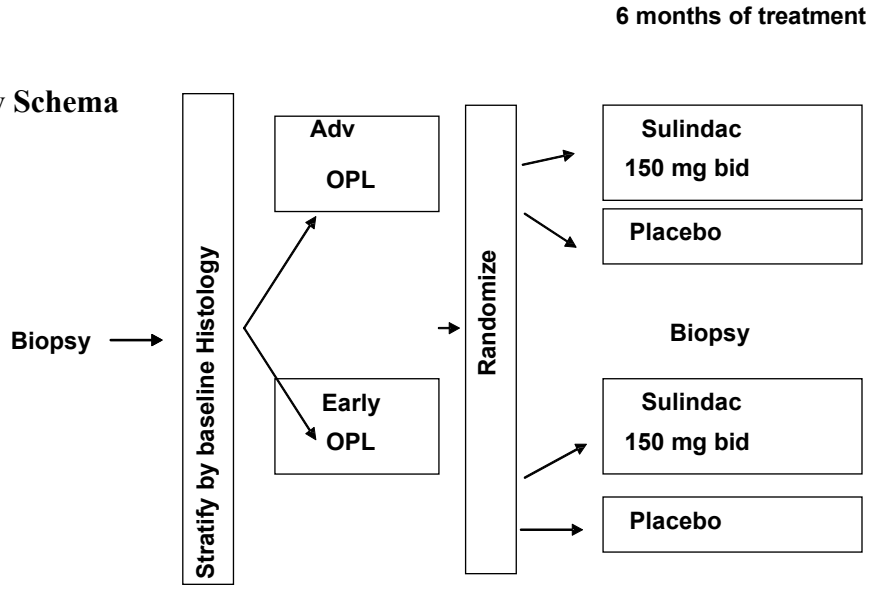
4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

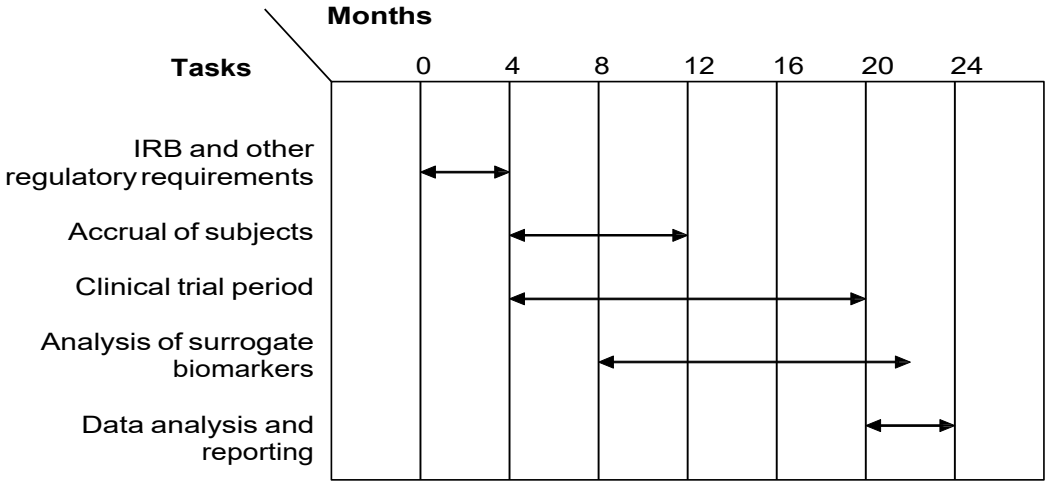
This is a pilot multi-center international double-blind, placebo-controlled randomized chemoprevention trial designed to determine the clinical efficacy of sulindac against OPL and biomarkers in OPL tissue. An informal, yet critical objective is to determine the feasibility of conducting such trials in India and the US. The targeted accrual is 177, with a prediction that 66, approximately 35-40% of the total number of participants consented, will be eligible and will be enrolled from AIMS, RCC and MSKCC. We expect however, that most subjects will be recruited from AIMS and RCC due to the substantially higher prevalence of this condition among the Indian compared to the US population. We intend on closing to recruitment in March of 2014 in order that we complete accrual of 66 randomized participants. All subjects will be required to meet study eligibility criteria and sign consent. All procedures will be performed in an outpatient setting.

After confirmation of the histology for the presence of either early premalignant lesions (hyperplasia at high risk sites, atypical hyperplasia, atypical hyperkeratosis, mild dysplasia) or advanced premalignant lesions (moderate or severe dysplasia), subjects will be stratified by clinic site and lesion grade (early or advanced lesion) subjects will be stratified by clinic site and for the presence of either early premalignant lesions (hyperplasia at high risk sites, atypical hyperplasia, atypical hyperkeratosis, mild dysplasia) or advanced premalignant lesions (moderate or severe dysplasia). Subjects will then be randomized to either sulindac 150 mg bid or placebo bid for 24 weeks. At the end of 24 weeks of treatment the lesion will be measured for the primary assessment of clinical efficacy, and biopsied for assessment of secondary endpoint biomarkers. Refer to Section 14 for the statistical analysis for primary and secondary endpoints. The study is powered to achieve an 80% chance of identifying a 30% response rate. The study is planned to accrue for 24 months and to be completed in 36 months.

Study Schema



Project Timeline



4.3 Intervention

Participants will be randomized to receive either sulindac 150 mg bid or placebo bid for 24 weeks. Sulindac, a pan-COX inhibitor, trademarked Clinoril by Merck, is an NSAID approved for arthritis but known to prevent colon polyps in humans and widely studied for cancer prevention through the NCI. It has been proven effective as a chemopreventive agent in several animal models of chemically induced cancer (11-13), and has been tested in humans for the prevention of colon cancer (14). The principal toxicities are the potential for upper GI ulceration, and platelet dysfunction, and renal toxicity.

5.1 THERAPEUTIC/DIAGNOSTIC AGENTS

Name of Drug/Agent
Sulindac is a NSAID indene derivative designated chemically as (Z)-5-fluoro-2methyl-1-[[p-(methylsulfinyl)phenyl]methylene]-1H-indene-3-acetic acid. Sulindac is an FDA approved drug for treatment of signs and symptoms of arthritis in adults.

Chemical Properties.
Sulindac is (Z)-5-fluoro-2methyl-1-[[p-(methylsulfinyl)phenyl]methylene]-1H-indene-3-acetic acid. The empirical formula is $C_{20}H_{17}FO_3S$ and the molecular weight is 356.42. It is a yellow crystalline compound insoluble under the PH of 6. Sulindac is metabolized to the active sulfide by reversible reduction. It is also metabolized to the inactive sulfone by irreversible oxidation.

Dose Groups and Duration of Exposure
Sulindac 150 mg po bid x 24 weeks
Placebo bid x 24 weeks

Packaging
Study drug at dose levels of 150 mg bid, and placebo, will be packaged in bottles of 100 capsules each. Subjects will receive two bottles at Week 0 and Week 12, dispensed through the investigational pharmacy at AIMS, RCC or MSKCC.

Storage of Study Medication
Study medication boxes will be stored in locked cabinets and distributed according to the treatment assignment randomly generated by the MSKCC CRDB. The pharmacist will maintain a dispensing log and be responsible for drug accountability. Subjects will keep the pills in a cool, dry place.

Study Drug Administration
Subjects will be instructed to take one capsule in the morning and one capsule in the evening. If a dose is missed, subjects will be instructed not to take the missed dose at the next dosing period. Subjects will be reminded of the importance of drug compliance during the study at the time of clinic visit and during telephone contacts.

Supplier
NCI will provide investigational drug supplies (sulindac 150 mg capsules or matching placebo) in 100 count bottles.

Labeling of Clinical Supplies
Fisher Bioservices is contracted to label and ship the study drug. Two-part labels (one fixed and one tear-off label) will be used for this blinded study. Upon receiving the drug shipment, the pharmacist at AIMS, RCC and MSKCC will remove the black sticker affixed to the tear-off portion of the bottle label to identify the drug contents as active or placebo. The label containing the lot number located below the bar code will also be removed by the pharmacist prior to storing the drug. For more detail please see the Pharmacy Standard Operating Procedures (Appendix).

The fixed label will contain the following instructions: take one tablet orally twice a day as directed (written in English and Malayalam for AIMS and RCC subjects; in English for MSKCC subjects). The tear-off portion of the label (written only in English for all sites) identifying the drug as active or placebo will be removed from the bottle at the time of dispensing and attached to the DIDR

Dispensing

For AIMS: The AIMS pharmacist receives a prescription from the study PI denoting the study IRB number and "CRDB Medical Record Number" (see the Randomization SOP for further details). The AIMS pharmacist matches the CRDB MRN on the prescription with the CRDB MRN and randomized treatment assignment contained in the email sent directly from the randomization coordinator at MSKCC. Once the treatment assignment is known, the AIMS pharmacist extracts a bottle containing 100 capsules of sulindac or placebo from the study inventory. The AIMS pharmacist writes the patient initials and "CRDB MRN" on the fixed and tear-off portions of the bottle label. The tear-off label is placed in the drug inventory log. The AIMS pharmacist provides the drug to Dr. Kuriakose or designated study personnel in a blinded fashion. All participants and study personnel, with the exception of the AIMS pharmacist must remain blinded to treatment assignment. Study drug is provided to the participant who is instructed to take one capsule twice each day.

For RCC: The RCC pharmacist receives a prescription from the study PI denoting the study IRB number and "CRDB Medical Record Number" (see the Randomization SOP for further details). The RCC pharmacist matches the CRDB MRN on the prescription with the CRDB MRN and randomized treatment assignment contained in the email sent directly from the randomization coordinator at MSKCC. Once the treatment assignment is known, the RCC pharmacist extracts a bottle containing 100 capsules of sulindac or placebo from the study inventory. The RCC pharmacist writes the patient initials and "CRDB MRN" on the fixed and tear-off portions of the bottle label. The tear-off label is placed in the drug inventory log. The RCC pharmacist provides the drug to Dr. Sebastian or designated study personnel in a blinded fashion. All participants and study personnel, with the exception of the RCC pharmacist must remain blinded to treatment assignment. Study drug is provided to the participant who is instructed to take one capsule twice each day.

For MSKCC: The dispensing pharmacist receives a prescription denoting the study IRB number and MSKCC Medical Record Number (MRN). The dispensing pharmacist determines the randomized treatment assignment for each new participant by searching for the provided MRN in the CRDB. Patient initials and "MSKCC MRN" are written on the fixed and tear-off portions of the bottle label. The tear-off label is placed in the drug inventory log. The dispensing pharmacist provides the drug to the patient, MD, RN or RSA in a blinded fashion. All participants and study personnel, with the exception of the dispensing pharmacist, must remain blinded to treatment assignment. Study drug is provided to the participant who is instructed to take one capsule twice each day.

Drug Accountability

A DIDR will be maintained by the pharmacist or designee. The subject will be identified by the subject identification number and "CRDB MRN" for AIMS and RCC, or "MSKCC MRN" for MSKCC subjects. Drug supplies are to be used only in accordance with this protocol and under the supervision of the Principal Investigator.

Dose Reduction
There will be no dose reduction. Subjects will be evaluated on an individual basis. Rechallenge of a subject will be instituted only after the initial toxicity has been resolved to the satisfaction of the Principal Investigator. If toxicity recurs, the subject will be withdrawn from the study.

Compliance
At each clinic visit, subjects will be provided with a pill diary (see Appendix IV. Pill Diary and Adverse Event Form).

Evaluation of Compliance
Study drug compliance will be monitored as follows:
1. Pill count: The RSA will perform pill counts during the 4-weekly clinic visits. Results will be recorded on the 4-weekly evaluation forms.
2. Pill diary: At each 4-weekly clinic visit, the PI or RSA at AIMS, RCC or MSKCC will review the pill diary that has been completed, signed and dated by each subject. The PIs or RSA will sign and date the pill diary.

Definition of Study Drug Compliance
Although study drug compliance will be evaluated by both methods described above, the definition of study drug compliance is based solely on pill count. A patient will be deemed compliant if the average 4-weekly pill count ≥ 80% (ie ≤ 20% capsules remain at end of each 4-week period). If the patient has consumed less than 80% for second time, the patient will be terminated from the study. Counseling will be provided for first episode noncompliance.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

For this study an OPL is defined as a lesion which can include atypical hyperplasia, atypical hyperkeratosis, leukoplakia, and erythroplakia/erythro-leukoplakia. Histology MUST be confirmed by an MSKCC pathologist, for all participating sites. An OPL may be located in the oral cavity, oropharynx.

6.2 Subject Inclusion Criteria

- The subject has a histologically suspected or confirmed index oral premalignant lesion, 12mm or greater in size that has not been biopsied in the past 6 weeks. Each index lesion must be either:

  An EARLY premalignant lesion defined to be at high risk as indicated by the presence of at least one of the following: atypical cells, mild dysplasia, or hyperplastic leukoplakia of high-risk sites, lateral and ventral tongue and floor of mouth

  OR

An ADVANCED premalignant lesion defined as the presence of at least one of the following: moderate dysplasia or severe dysplasia (excluding CIS)

- The subject is > 18 years of age.
- The subject's life expectancy is > 12 weeks and Zubrod performance status is 0 or 1 (Appendix VIII).
- The subject meets the following laboratory eligibility criteria during a time not to exceed 4 weeks prior to randomization.
    - Hemoglobin level above the lower limit of normal.
    - WBC count > 3,000/µl.
    - Platelets count > 125,000/µl.
    - Total bilirubin ≤ 1.5 x ULN.
    - AST (SGOT) and ALT (SGPT) ≤ 2.5 x ULN
    - BUN and serum creatinine ≤ 1.5 x ULN.
- If the subject is female and of childbearing potential (women are considered not of childbearing potential if they are at least two years postmenopausal and/or surgically sterile), she:
    - has been using adequate contraception (abstinence, IUD, birth control pills, or spermicidal gel with diaphragm or condom) since her last menses and will use adequate contraception during the study, AND
    - is not lactating, AND
    - has a documented negative serum pregnancy test within 14 days prior to randomization.
- If the subject is male, will use adequate contraception during the study
- The subject's history/use of NSAIDs, aspirin, corticosteroids meets the following criteria:
    - total oral/intravenous corticosteroid use has been < 14 days within six months of the Baseline visit, and
    - total inhaled corticosteroid use has been < 30 days within six months of the Baseline visit, and
    - is willing to limit aspirin use to ≤ 150 mg po per day (typical cardioprotective dose in India) or ≤ 81 mg po per day (typical cardioprotective dose in the US) for the duration of the study, and is willing to abstain from chronic use of all NSAIDs and COX-2

inhibitors for duration of study. Chronic use of NSAIDs is defined as a frequency of ≥ 3 times/week AND for more than a total of 14 days a year.

- The subject has discontinued any other chemopreventive therapy at least 3 months prior to the Baseline visit and all toxicities have been fully resolved.
- The subject has signed and dated the informed consent statement.
- The subject is willing and able to fully participate for the duration of the study.
- If applicable, the subject has been counseled on smoking cessation.

6.3 Subject Exclusion Criteria

- The subject has had chemotherapy, immunotherapy, hormonal therapy (other than HRT for menopause), or radiation therapy within 3 weeks of the Baseline visit.
- The subject has not recovered from the acute toxic effects of chemotherapy, immunotherapy, hormonal therapy, or radiation therapy.
- The subject will need concurrent chemotherapy, radiotherapy, hormonal (other than HRT for menopause), or immunotherapy during the time of study.
- The subject has a history of hypersensitivity to sulindac, COX-2 inhibitors, NSAIDs, salicylates.
    - History of myocardial infarction, angina, or coronary artery disease within the past 6 months, or active cardiac disease.
    - The subject is of New York Heart Association (NYHA) Class 3 or 4 cardiac status as defined below:
      New York Heart Association (NYHA) Classification: A Functional and Therapeutic Classification for Prescription of Physical Activity For Cardiac Patients.
        - Class I: patients with no limitation of activities; they suffer no symptoms from ordinary activities.
        - Class II: patients with slight, mild limitation of activity; they are comfortable with rest or with mild exertion.

- Class III: patients with marked limitation of activity; they are comfortable only at rest.
- Class IV: patients who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

- The subject has been diagnosed with or has been treated for esophageal, gastric, pyloric channel, or duodenal ulceration.
- The subject has a history of invasive cancer within the past 1 (one) year (excluding non-melanoma skin cancer and in situ cervical cancer).
- The subject has a chronic or acute renal or hepatic disorder or a significant bleeding disorder or any other condition which, in the Institutional Principal Investigator's opinion, might preclude study participation.
- The subject has a past history of or active inflammatory bowel disease (eg. Crohn's disease or ulcerative colitis) or pancreatic disease.
- The subject has received any investigational medication within 30 days of the Baseline visit or is scheduled to receive an investigational drug during the course of the study.
- The subject is, in the opinion of the Institutional Principal Investigator, not an appropriate candidate for study participation.
- The subject participated in the study previously and was withdrawn.

Screen Failure. All subjects considered for participation and found ineligible must be recorded in the Screen Failure Log. Subject confidentiality is maintained, but reason for exclusion is required.

7.0 RECRUITMENT PLAN

In Cochin and Trivandrum, Kerala, India, where OPL and HNSCC are a significant clinical problem, subjects will be recruited from the AIMS Head and Neck and Dental Services and from the RCC the Head and Neck Surgical Service of the Surgical Oncology Department. In addition, subjects from Cochin suburban communities will be recruited; the AIMS institute has an existing outreach program to these communities in which patients are able to be screened and receive care in their homes. In New York City, New York, subjects will be recruited from the subject population of the Head and Neck Service, MSKCC, the coordinating center of this pilot multi-center international chemoprevention trial. We expect to enroll six eligible participants at MSKCC, 30 eligible participants at AIMS, and 30 eligible participants at RCC.

Upon assessment of accrual in March 2012, we predict recruitment of 66 randomized subjects will be completed in two years, and therefore will close the study to recruitment to accrual in 2014.

The RSA will circulate mailings to local referring professionals to solicit referrals for study consideration. Subjects of all age, sex, and ethnic groups are to be considered however we anticipate that >90% of the study population will be Indian, due to the substantially higher prevalence of OPL among the Indian compared to the US population. After identification of a candidate OPL, and review of the inclusion and exclusion criteria, subjects will be approached by the consenting professional regarding candidacy for the trial. Interested subjects will review the subject information package, be orally counseled regarding the trial, questions answered and then be asked to sign the consent form. Consented subjects will then be registered at MSKCC. Biopsy of the lesion will be performed to confirm histologic eligibility and stratification. Subject registration at MSKCC will then be completed, to allow for randomization at MSKCC to occur.

Subjects will not be responsible for study related expenses. AIMS and RCC subjects will be provided with a travel reimbursement of $7 (350 rupees) at each visit. Therefore total reimbursement will be $56 for the eight visits. As per MSKCC standard procedures, MSKCC subjects will not be reimbursed for their participation in this trial.

8.1 PRETREATMENT EVALUATION

The Pretreatment Period consists of the Baseline (or Week -2) and Week 0 visits, during which no study medication is taken. The time that elapses between these two visits should not exceed eight weeks. At the Baseline visit written informed consent will be obtained for all potential study participants. To properly document that informed consent was obtained the date the informed consent was signed must be recorded in the subject's medical record. A copy of the signed informed consent must be provided to the subject.

8.2 Baseline

At the Baseline visit, a complete alcohol and tobacco history, inclusion/exclusion criteria, laboratory tests including a pregnancy test (if required), medical history and physical exam will be performed and recorded in the medical record.

Physical Exam to include: vital signs, height, weight, blood pressure (sitting), temperature

Alcohol and tobacco history will be recorded directly onto CRF.

Stage One of Registration (See Section 15.1)

Clinical Laboratory Tests and Other Specialized Tests and Procedures

The following will be performed at the Baseline visit:

Clinical laboratory tests to include:

- CBC with differential
- Platelet count
- BUN
- Creatinine
- AST (SGOT)
- ALT (SGPT)
- Alkaline phosphatase
- Uric acid
- Glucose
- Total protein
- Total bilirubin
- Pregnancy (if applicable)

Urinalysis to include:

- pH
- Specific gravity
- Glucose
- Ketones
- Protein
- WBC
- RBC
- Bacteria
- Casts

Color photograph of all lesions. For monitoring purposes, the sample number will be recorded on each photograph.

Record bi-dimensional measurements for all oral premalignant lesions. The index lesion will be measured before and after biopsy to ensure lesion is at least 5mm after biopsy.

Biopsy lesion(s)

The baseline biopsy of the index lesion (or lesion area) will be bisected. However, should it be clinically indicated to biopsy more than one lesion, ALL biopsies must be sent for local pathology evaluation. The pathology containing the most advanced grade will be used as the INDEX lesion which will be followed for purposes of this study. Only ONE index lesion is allowed.

For AIMS subjects: One half of the baseline biopsy of the index lesion will be immediately placed into a labeled Eppendorf screw cap cryotube (glove handling for

RNAase precaution) and placed in liquid nitrogen. The cryotube will then be hand-delivered to Dr. Elango's laboratory where cDNA will be prepared, placed in a second labeled Eppendorf screw cap cryotube and stored at -80°C until transported to WMC, NY (See Appendix V. Shipping Instructions) for COX-2 mRNA analysis. The other portion of the baseline biopsy will be placed in a pre-labeled bottle of formalin and sent to the AIMS Department of Pathology where the H and E stained section of the biopsy will be evaluated and the lesion diagnosed as either early or advanced grade according to standard criteria. The AIMS pathologist will make photomicrographs of the biopsy and electronically transmit them to the Department of Pathology, MSKCC for confirmation of grade. The MSKCC pathologist may confer with the AIMS pathologist if there is disagreement. In the unlikely event the images are not satisfactory; the MSKCC pathologist will request overnight delivery of a single H&E slide from India. The MSKCC pathologist's decision will be final. The pathology containing the most advanced grade will be used as the INDEX lesion which will be followed for purposes of this study. Only ONE index lesion is allowed.

For RCC subjects: One half of the baseline biopsy of the index lesion will be immediately placed into a labeled Eppendorf screw cap cryotube (glove handling for RNAase precaution) and placed in liquid nitrogen. The cryotube will then be stored at -80 degree Celsius, until shipment on dry ice once a week is made to Dr. Elango's laboratory where cDNA will be prepared, placed in a second labeled Eppendorf screw cap cryotube and stored at -80°C until transported to WMC, NY (See Appendix V. Shipping Instructions) for COX-2 mRNA analysis. The other portion of the baseline biopsy will be placed in a pre-labeled bottle of formalin and sent to the RCC Department of Pathology where the H and E stained section of the biopsy will be evaluated and the lesion diagnosed as either early or advanced grade according to standard criteria. The RCC pathologist will make photomicrographs of the biopsy and electronically transmit them to the Department of Pathology, MSKCC for confirmation of grade. The MSKCC pathologist may confer with the RCC pathologist if there is disagreement. In the unlikely event the images are not satisfactory; the MSKCC pathologist will request overnight delivery of a single H&E slide from India. The MSKCC pathologist's decision will be final. The pathology containing the most advanced grade will be used as the INDEX lesion which will be followed for purposes of this study. Only ONE index lesion is allowed.

For MSKCC subjects: One half of the baseline biopsy of the index lesion will be immediately placed into a labeled Eppendorf screw cap cryotube (glove handling for RNAase precaution) and placed in liquid nitrogen. The cryotube will then be hand-delivered to Dr. Dannenberg's laboratory where cDNA will be prepared, placed in a second labeled Eppendorf screw cap cryotube and stored at -80°C until COX-2 mRNA analysis. The other portion of the baseline biopsy will be sent to the MSKCC Department of Pathology for routine histology evaluation. As for the AIMS and RCC subjects described above, the pathology containing the most advanced grade will be used

as the INDEX lesion which will be followed for purposes of this study. Only ONE index lesion is allowed.

The MSKCC pathologist will electronically transmit the pathology confirmation for AIMS, RCC, and MSKCC subjects to the MSKCC RSA who will document this is in Part Two of the eligibility form and submit to the PPA to complete the second phase of registration. Once the registration process with the PPA system has been completed, randomization will occur.

Record concomitant medications (any use of a nicotine supplement, ie. patch or gum, herbal supplements and vitamins are considered a medication and should be recorded).

Record adverse signs and symptoms.

Tissue banking blood: Collect one 5 mL lavender top tube of blood

For AIMS subjects: Blood will be hand-delivered to Dr. Elango's laboratory and aliquoted into 3-4 labeled Eppendorf screw cap cryotubes and stored at -80°C until transported to MSKCC, NY (See Appendix V. Shipping Instructions) for future genotyping analysis.

For RCC subjects: Blood will be aliquoted into 3-4 labeled Eppendorf screw cap cryotubes and stored at -80°C and will be sent in batches, once a week to Dr. Elango's laboratory for future transport to MSKCC, NY (See Appendix V. Shipping Instructions) for future genotyping analysis.

For MSKCC subjects: The RSA will email members of the Molecular Epidemiology Laboratory when blood will be collected for a study participant (denote study ID number) and Protocol #04-099. Once the blood is collected, the specimen will be hand-delivered to one of the lab members or deposited in the drop box outside the Molecular Epidemiology Lab (Schwartz 732). Blood will be aliquoted into 3-4 labeled Eppendorf screw cap cryotubes and stored at -80°C for future genotyping analysis. Cryotubes will be labeled with the prefix SUL and the four digit sample number.

Smoking and tobacco cessation counseling provided.

8.3 Admission of Subjects

The following procedures will occur at the Week 0 visit:

Documentation of **smoking cessation counseling** completion must be recorded in subject's medical record.

Pregnancy test: If not already completed 14 days prior to Week 0 visit, order STAT for results to be available prior to randomization.

Record any new interim concomitant medications

Stage Two of Registration (See Section 15.1)

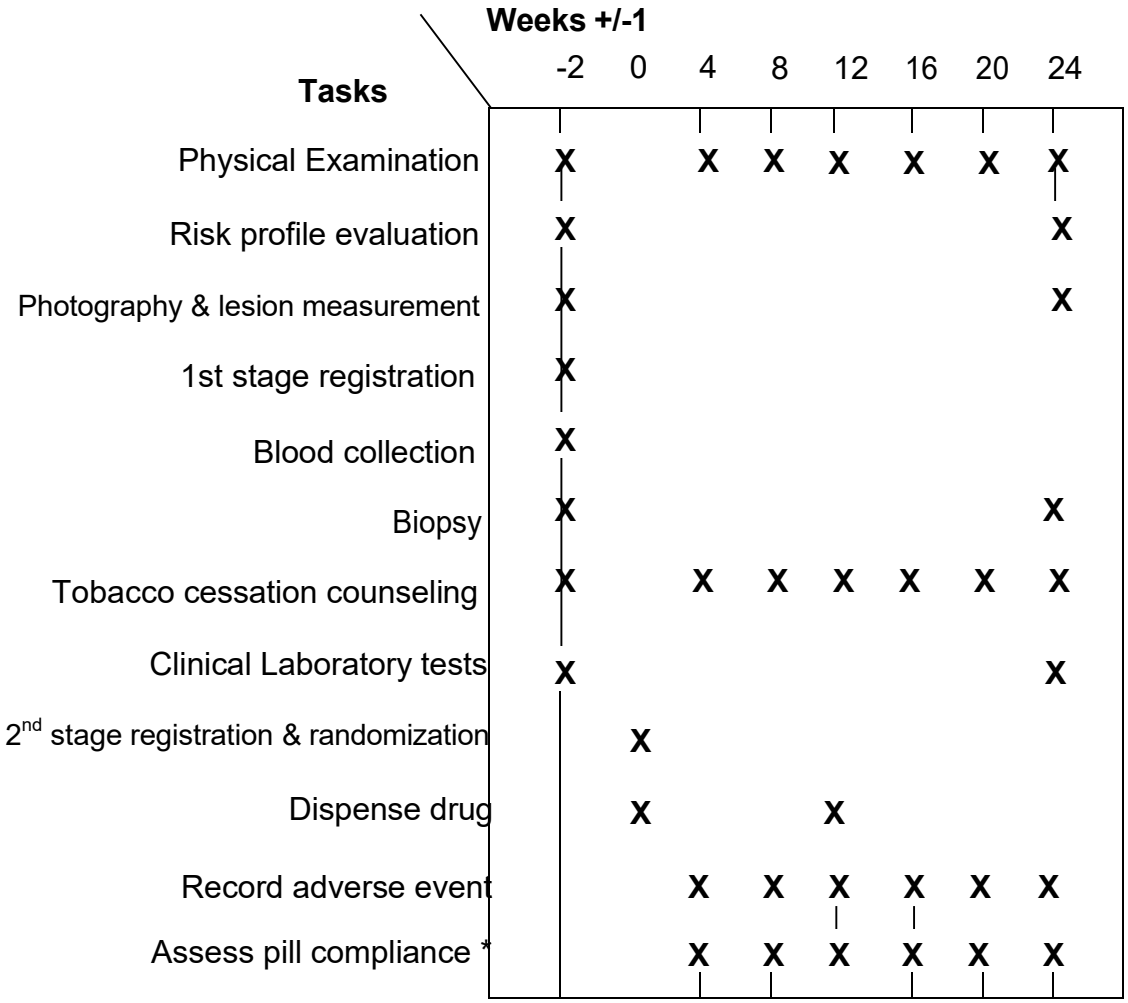
Randomization (See Section 15.2)

Dispense Study Medication: See Section 5 and the Pharmacy Standard Operating Procedures.

9.0 TREATMENT/INTERVENTION PLAN

SCHEMA

Study schema for individual subjects



* Review pill diary and determine compliance using pill count (>= 80%)
Monthly follow-up phone calls will also be performed at Weeks 2, 6, 10, 14, 18 and 22

10.1 EVALUATION DURING TREATMENT/INTERVENTION

Subject Status and Dosing Schedule
Once the subject is randomized they should begin their first dose of study medication by the subsequent morning. Each subject will be instructed to continue taking the dispensed study medication twice a day for the duration of the 24 week Treatment Period.

Procedures and Observations
Study Medication Administration
The subject will return for visits at Weeks 4, 8, 12, 16, 20 and 24 with their previously dispensed study medication bottle, pill diary and remaining pills.
Record Concurrent Medication

Compliance

Subject compliance is an important aspect of clinical trials. It is well documented that subjects recruited to studies performed in India recognize that study participation can offer access to quality health care and medications that may not be otherwise affordable. As a result, subjects exhibit a high level of compliance and motivation to attend all required study visits and ingest study drug as per protocol. *Importantly, an independent study by a global Clinical Research Organization (CRO) concluded that India has one of the best subject return rates and compliance to clinical trial protocols in the world (Applied Clinical Trials, February 2003).*

Subject compliance will be assessed/encouraged using three methods:

1. **Pill diary**
2. **Pill count**
3. **Four-weekly phone calls (approximately 2 weeks after each clinic visit)**

1. Patients will be provided and instructed on the use of a **pill diary** (See Appendix IV) that will be completed daily, signed and dated by the patient. At 4-weekly clinic visits, research personnel will monitor the patient's compliance, sign and date the pill diary. Signed and dated pill diaries collected in India will be immediately faxed to MSKCC for review and filing. In addition, a copy of the pill diary will be sent to MSKCC for monitoring and auditing, in batches as appropriate.

2. At each clinic visit, patients will return any unused study medication and the study medication bottle to the investigators, who will conduct a **pill count**. If the patient has consumed less than 80% for the second time, the patient will be terminated from the study. Counseling will be provided for first episode noncompliance.

3. **Four-weekly phone calls**: patients will be contacted by research personnel approximately two weeks after each 4-weekly clinic visit. Patients will be encouraged to maintain compliance and be asked about any adverse effects or concerns they might have. It is important to note that our Indian collaborators have confirmed that most of their patients can be accessed through telephone - most directly in their homes, although some may need to be contacted through local telephone offices. Our collaborators assure us that follow up through telephone is certainly possible.

Below is the outline of the schedule of events that will take place during the Treatment Period.

Treatment Period 4 Weeks (±1 Week)
Physical exam
Record concomitant medication.
Record adverse signs and symptoms.
Record in subject's medical record.
Pill diary and pill count compliance assessment. Verbal counseling for less than 80% compliance.
Tobacco cessation counseling

Treatment Period 8 Weeks (±1 Week)
Physical exam.
Record concomitant medication.
Record adverse signs and symptoms.
Pill diary and pill count compliance assessment. If less than 80% for second time, terminate from the study. Counseling for first episode noncompliance.
Tobacco cessation counseling

Treatment Period 12 Weeks (-1 Week or +2 Weeks)
Physical exam.
Record concomitant medication.
Record adverse signs and symptoms.
Pill diary and pill count compliance assessment
Dispense drug for 12 weeks of treatment
Tobacco cessation counseling

Treatment Period 16 Weeks (±1 Week)
Physical exam
Record concomitant medication.
Record adverse signs and symptoms.
Pill diary and pill count compliance assessment
Tobacco cessation counseling

Treatment Period 20 Weeks (±1 Week)
Physical exam
Record concomitant medication.
Record adverse signs and symptoms.
Pill diary and pill count compliance assessment
Tobacco cessation counseling

Treatment Period 24 Weeks (-1 Week or +2 Weeks)
Physical exam to include: vital signs, weight, blood pressure (sitting), temperature
Color photograph all lesions prior to biopsy of index lesion.
Provide bi-dimensional measurements for all lesions (if possible).
Pill diary and pill count compliance assessment
Biopsy index lesion.

Do not bisect tissue for COX-2 mRNA analysis (Baseline Only).
See **Page 31** for Standard Biopsy Procedures.
Should it be clinically necessary to biopsy any additional lesions, ALL biopsies must be sent for local pathology evaluation.
Send specimen for local pathology evaluation.

Laboratory to include:

- CBC with differential
- Platelet count
- BUN
- Creatinine
- AST (SGOT)
- ALT (SGPT)
- Alkaline phosphatase
- Uric acid
- Glucose
- Total protein
- Total bilirubin
- Urinalysis to include:
- pH
- Specific gravity
- Glucose
- Ketones
- Protein
- WBC
- RBC
- Bacteria
- Casts

Record all concomitant medications.
Record all adverse signs and symptoms.
Pill diary and pill count compliance assessment

Tobacco cessation counseling

Withdrawal of a Subject Prior to Study Completion
Every effort will be made by the Principal Investigator to keep the subject in the study; however, should the subject decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. Data will include a complete final evaluation at the time of the subject's withdrawal with an explanation of why the subject is terminated from treatment.

If, for any reason, a subject withdraws before they have completed the study, the reason for withdrawal must be entered on the CRF.

Study Procedures

Measurement of lesions (Baseline and Week 24)
Obtain the bi-dimensional measurements for all the oral premalignant lesion(s) and record on CRF. For example:
Lesion #1 2 mm width x 3 mm length = 6 mm^2 (product)
Lesion #2 1 mm width x 3 mm length = 3 mm^2 (product)
Sum of products = 9 mm^2 (6 mm^2 + 3 mm^2)
The pre-biopsy measurement of the index lesion should be recorded on the appropriate Week 24 Oral Evaluation CRF.

Color Photographs of All Lesions
Color photographs or slides (per institutional standards) will be obtained for all lesions primarily for the documentation of clinical response.

Standard Biopsy Procedures
Note: Only the Baseline biopsy specimens will be bisected.
Local anesthetic: at the discretion of the surgeon, topical application of 20% benzocaine, and/or injection of 1 or 2% lidocaine with or without 1:100,000 epinephrine or 3% mepivacaine without epinephrine.

Incisional biopsy (4 mm punch) of the oral leukoplakia or erythroplakia lesion: using a Baker 4 mm punch, the center or the clinically most suspicious area of the lesion is biopsied with a twisting motion. The circular punched out tissue is grasped gently with fine toothed forceps and excised just deep to the submucosa with the #15 scalpel. Care is taken to avoid collecting significant amounts of fat or muscle and to avoid crushing the specimen.

The *Baseline sample* is placed mucosa side down on a clean hard surface such as a piece of wax paper and immediately bisected with a scalpel.

For AIMS subjects: One half of the baseline biopsy of the index lesion will be immediately placed into a labeled Eppendorf screw cap cryotube **(**glove handling for RNAase precaution**)** and placed in liquid nitrogen. The cryotube will then be hand-delivered to Dr. Elango's laboratory where cDNA will be prepared, placed in a second labeled Eppendorf screw cap cryotube and stored at -80°C until transported to WMC, NY (See Appendix V. Shipping Instructions) for COX-2 mRNA analysis. The other portion of the baseline biopsy will be placed in a pre-labeled bottle of formalin and sent to the AIMS Department of Pathology where the H and E stained section of the biopsy will be evaluated and the lesion diagnosed as either early or advanced grade according to standard criteria. The AIMS pathologist will make two photomicrographs of the biopsy and electronically transmit them to the Department of Pathology, MSKCC for confirmation of grade. The MSKCC pathologist may confer with the AIMS pathologist if there is disagreement. In the unlikely event the images are not satisfactory; the MSKCC pathologist will request overnight delivery of a single H&E slide from India. The MSKCC pathologist's decision will be final. The pathology containing the most advanced grade will be used as the INDEX lesion which will be followed for purposes of this study. Only ONE index lesion is allowed.

For RCC subjects: One half of the baseline biopsy of the index lesion will be immediately placed into a labeled Eppendorf screw cap cryotube **(**glove handling for RNAase precaution**)**, snap-frozen in liquid nitrogen, and stored at -80°C. Weekly dry ice shipment of biopsies will be made to Dr. Elango's lab at AIMS and stored -80°C until transported to WMC, NY (See Appendix V. Shipping Instructions) for COX-2 mRNA analysis. The other portion of the baseline biopsy will be placed in a pre-labeled bottle of formalin and sent to the AIMS Department of Pathology where the H and E stained section of the biopsy will be evaluated and the lesion diagnosed as either early or advanced grade according to standard criteria. The AIMS pathologist will make two photomicrographs of the biopsy and electronically transmit them to the Department of Pathology, MSKCC for confirmation of grade. The MSKCC pathologist may confer with the AIMS pathologist if there is disagreement. In the unlikely event the images are not satisfactory; the MSKCC pathologist will request overnight delivery of a single H&E slide from India. The MSKCC pathologist's decision will be final. The pathology containing the most advanced grade will be used as the INDEX lesion which will be followed for purposes of this study. Only ONE index lesion is allowed.

For MSKCC subjects: One half of the baseline biopsy of the index lesion will be immediately placed into a labeled Eppendorf screw cap cryotube **(**glove handling for RNAase precaution**)** and placed in liquid nitrogen. The cryotube will then be hand-delivered to Dr. Dannenberg's laboratory where cDNA will be prepared, placed in a second labeled Eppendorf screw cap cryotube and stored at -80°C until analyzed for COX-2 mRNA. The other portion of the baseline biopsy will be sent to the MSKCC Department of Pathology for routine histology evaluation. As for the AIMS subjects described above, the pathology containing the most advanced grade will be used as the INDEX lesion which will be followed for purposes of this study. Only ONE index lesion is allowed.

For *week 24 biopsies* the entire specimen is placed in formalin and sent to pathology for histologic evaluation.

Hemostasis is achieved by direct pressure, silver nitrate, or suture.

Subjects are provided with oral wound care instructions: 4-6 times daily local rinsing with saline and standard post surgical instructions including notification of consulting professional for heavy bleeding, severe pain, or fever.

For analgesia, subjects will be told to take acetaminophen 650 mg po qid PRN.

Test Type	Time 1	Non-Billable Research Charges	Time 2	Non-Billable Research Charges	Time 3	Non-Billable Research Charges
Transoral biopsy of index pre-malignant lesion	Intial clinic visit	None	Week 24 clinic visit	**MSKCC pts: 10 bx @ $400 = $4,000**		**MSKCC pts: 10 bx @ $400 = $4,000**
Blood and Urine Clinical Laboratory Tests	Intial clinic visit	None	Week 24 clinic visit	**MSKCC pts: 10 pt @ $300 = $3,000**		

11.0 TOXICITIES/SIDE EFFECTS

This agent has been used extensively in humans with an excellent safety profile. All subjects will be closely monitored for signs and symptoms of toxicity. Toxicities will be reported immediately as outlined in the DSM plan. Toxicities will be graded according to the National Cancer Institute Common Toxicity Criteria.

The most frequent adverse reactions occurring with sulindac are GI including pain in 10%, dyspepsia, nausea with or without vomiting, diarrhea, constipation, flatulence, and cramps. Rash, Dizziness, tinnitus, and edema can occur in 1% of subjects. Less than 1% of subjects may have GI ulceration, jaundice, hepatitis, biliary stones, skin reactions, CHF, thrombocytopenia, hematuria, hyperkalemia, vertigo, depression, or epistaxis.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Clinical Response (at Week 24)
Complete Response (CR): Disappearance of all evidence of the lesions.
Partial Response (PR): 50% or greater decrease in the product of the perpendicular diameters of the index lesion. No lesion may increase in size and no new lesion may appear.
No Change (NC): No change in the size of the lesion(s) and no new lesions appearing.
Progressive Disease (PD): Any increase > 25% in the product of the perpendicular diameters of the index lesion or in the estimated size of non-measurable lesions or the appearance of an unequivocal new lesion or progression to invasive carcinoma.

Histologic Response (at Week 24):
Complete Response (CR): Complete reversal of dysplasia to normal epithelium in index lesion and all other premalignant lesions.
Partial Response (PR): Improvement of the degree of dysplasia in the selected premalignant index lesion from advanced to early or from early to normal epithelium with no change in non-index lesions.
No Change (NC): No change in the degree of dysplasia in the selected lesion.
Progressive Disease (PD): Any increase in the severity of grade of histology or progression to a carcinoma in the premalignant index lesion.

Definition of Final Response:
To account for possible discordance between clinical and histologic responses the following definitions of final response will be used, where CR=Complete response, PR=Partial response, NC=No Change, and PD=Progression of disease:

Baseline EARLY premalignant lesion		***Clinical***			
		CR	***PR***	***NC***	***PD***
Histological	***CR***	CR	PR	PR	PD
	PR	PR	PR	NC	PD
	NC	PR	PR	NC	PD
	PD	PD	PD	PD	PD

Baseline ADVANCED Premalignant lesion		*Clinical*			
		CR	***PR***	***NC***	***PD***
Histological	***CR***	CR	PR	PR	PD
	PR	PR	PR	PR	PD
	NC	PR	NC	NC	PD
	PD	PD	PD	PD	PD

13.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the subject develops progressive disease he/she will be taken off study and referred for alternative therapy.

If at any time the subject develops unacceptable toxicity he/she will be removed from study.

If at any time the subject is found to be ineligible for the protocol as designated in the section on Criteria for Subject Eligibility (i.e., a change in diagnosis), the subject will be removed from the study.

Subjects will also be immediately discontinued from this study for any of the following reasons:

Subject fails to adhere to the conditions outlined within the Schedule of Observations and Procedures. This could include failure of the subject to report for scheduled examinations, treatments, biopsy procedures or the use of excluded medication.

Development of an invasive or progressive disease. Invasive carcinoma discovered at any time during study will be considered progressive disease (PD) and will result in removal from the study and referral for appropriate treatment.

Any new lesion (other than the index lesion) that, in the opinion of the Investigator, warrants a biopsy for histologic review.

The development of an AE with a grade 3 or 4 toxicity.

Intercurrent illness necessitating premature termination.

Subject withdraws consent.

At the Principal Investigators' discretion, the subject is removed from the study.

Serious adverse event thought to be related to study drug.

14.0 BIOSTATISTICS

Sample Size Justification

This is a pilot multi-center international double-blind, placebo-controlled randomized study of sulindac, a pan-COX inhibitor, in subjects with oral premalignant lesions treated at AIMS and RCC, India and MSKCC, NY. Sixty six subjects will be randomized to receive either sulindac or placebo for 24 weeks.

The primary objective of this study is to evaluate and compare the final response rates (CR or PR based on clinical and histological criteria evaluated at Week 24) in the sulindac and placebo arms. As this is a pilot study, we do not have preliminary information on the expected response rate in the placebo arm of this high risk population in India, nor on what magnitude of difference in response rates we might expect to see between the two arms. Based on clinical experience with similar subjects in the USA we expect the response rate in the placebo arm will be approximately 4%. After accounting for a 10% dropout rate, with 33 subjects in each arm we will be able to detect a difference of 30% between response rates in the two arms with 80% power and a significance level of .05. This study will be used to decide whether sulindac is worth studying further as a chemopreventive agent for HNSCC and will provide preliminary information that is necessary to design a more definitive chemoprevention trial.

Analysis of Efficacy Endpoints

Primary Efficacy

The primary efficacy endpoint for this study is the percentage of subjects with CR or PR based on the final response evaluation at Week 24. The estimates of the percentages in each arm will be given together with 95% confidence intervals. The percentages will be formally compared using the Mantel-Hantel statistic stratifying on early/advanced premalignant lesions in an intent-to-treat analysis. Baseline demographic information and other potential prognostic factors will be explored using generalized linear models.

Secondary Efficacy

Response rates in the early and advanced premalignant subpopulations will be studied descriptively by estimating the percentages and their confidence intervals. Separate descriptive analyses will be done using clinical response and histological response as endpoints and proceeding as described above for the primary efficacy endpoint. Descriptive analyses will also assess the possible correlation between the clinical and histological response.

Analysis of Biomarkers

Descriptive statistics will be used to assess the effects of sulindac on modulation of the biomarkers. Correlations among the biomarkers and between the biomarkers and response rate will be evaluated by both tabular and graphical form. The joint effects of biomarker expression on response status may be explored through the use of classification and regression trees such as CART or logistic regression.

Analysis of Safety Endpoints

All adverse events will be descriptively summarized. These analyses will consist of displays of the distribution by treatment group and disease category of the numbers of subjects reporting at least one episode of a specific adverse event (incidence table), the total number of episodes for each event reported (frequency table), and the severity and attribution to study drug of each episode reported (severity and attribution table).

Listings of adverse events by subjects and by event will include the time to onset, the duration of each event, and the Principal Investigator's opinion of the relationship of the event to study medication, whether it was a serious event, and whether it caused withdrawal.

Adverse events will be coded using the World Health Organization (WHO) dictionary. Pearson's chi-square will be used to test the association of treatment and overall incidence within each preferred term and body system. The proportion of subjects withdrawn due to adverse events will also be compared.

At Week 24, clinical laboratory data for each treatment will be summarized for change from Pretreatment to Posttreatment visits. Incidence of clinically significant laboratory test results will be compared between treatment groups. Each laboratory value will be examined descriptively.

Shifts in laboratory test values will be compared among treatments in terms of numbers of subjects showing an increase, decrease, and no change, with respect to the normal range, using a chi-square test. Changes from Baseline (calculated as the value at Week 24 minus the Baseline value) will also be compared.

Laboratory data obtained any time after the first dose of study medication will not be used as Baseline data. Similarly, laboratory data that are obtained more than seven days after the last dose of study medication will be excluded from the shift table analyses.

Changes in weight and vital signs from Pretreatment to Post-treatment will be calculated and compared across treatment groups using a t-test or a Wilcoxon rank-sum test.

The percentage of subjects showing changes from Baseline in the laboratory tests defined below will be compared between treatment groups:

SGOT (AST) and SGPT (ALT):	> 3 x ULN (upper limits of normal value)
Alkaline Phosphatase:	> 1.25 x ULN
Total Bilirubin:	> 1.5 x ULN
Creatinine:	> 1.3 x ULN
BUN:	> 2.0 x ULN
Hematocrit:	a decrease > 5 percent points (relative to subject's Baseline value)
Hemoglobin:	a decrease > 2 grams/dl (relative to subject's Baseline value)
White Blood Cells:	< 3000/ul
Platelets:	< 100,000/ul

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. The PPR fax numbers are (646) 735-0008 and (646) 735-0003. Registrations can be phoned in or faxed. The completed signature page of the written consent/verbal script and a completed Eligibility Checklist must be faxed to PPR.

Stage two:
Clinical Laboratory Test Results:
For MSKCC subjects: the RSA at MSKCC will obtain the results of the clinical laboratory tests from the subject's electronic medical record. The RSA will record the values on the CRF and Part Two of the eligibility form. The completed eligibility form will be faxed to the PPR at MSKCC once the pathology result from the baseline biopsy of the oral premalignant lesion is known. A re-consent is required if registration to part 2 exceeds the 30 day limit.

Pathology Results:

The MSKCC pathologist will electronically submit the pathology of the baseline biopsy from the index lesion for subjects from all sites to the RSA at MSKCC. The RSA will document the histology in Part Two of the eligibility form and submit the completed eligibility form to the PPR to complete the second phase of registration. Once the registration process with the PPR system has been completed, randomization will occur.

15.1.1 For Participating Sites:

Central registration for this study will take place at Memorial Sloan Kettering Cancer Center (MSKCC).

To complete registration and enroll a participant from another institution, the study staff at that site must contact the designated research staff at MSKCC to notify him/her of the participant registration. The site staff then needs to fax registration/eligibility documents to the Department of Surgery at MSKCC at 212-557-0495

Part 1 Registration
The following documents must be sent for each enrollment **within 24 hours** of the informed consent form being signed:

- The completed or partially completed MSKCC eligibility checklist
- The signed informed consent and signed HIPAA Authorization form
- Supporting source documentation for eligibility questions (laboratory results, pathology report, radiology reports, MD notes, physical exam sheets, medical history, prior treatment records, and EKG report).

Upon receipt, the research staff at Memorial Sloan Kettering Cancer Center will conduct an interim review of all documents. At Part 1 registration, subject will be registered as PENDING until clinical laboratory and pathology reports are received within 30 days of the consent.

Part 2 Registration

Clinical Laboratory Test Results:

The RSA at AIMS and RCC will obtain the results of the clinical laboratory tests from their respective clinical laboratories. The RSA will record these values on the CRF and Part Two of the eligibility form. The eligibility form will be faxed to the RSA at MSKCC (011-212-557-0495) who will then submit it to PPR at MSKCC once the Pathology result from the baseline biopsy of the oral premalignant lesion is known. The fully completed eligibility checklist, source documentation for both laboratory and pathology results, and MSKCC pathologist e-mail confirmation of histology must be faxed to the PPR for part 2 registration within 30 days of initial registration. A re-consent is required if registration to part 2 exceeds the 30 day limit.

If the eligibility checklist is complete, participant meets all criteria, all source documentation is received, the participating site IRB has granted approval for the protocol, and the site is in good standing with MSKCC, the MSKCC research staff will send the completed registration

documents to the MSKCC Protocol Participant Registration (PPR) Office to be enrolled as stated in section 15.1. The participant will be registered.

Once eligibility has been established and the participant is registered, the participant will be assigned an MSKCC Clinical Research Database (CRDB) number (protocol participant number). This number is unique to the participant and must be written on all data and correspondence for the participant. This protocol participant number will be relayed back to study staff at the registering site via e-mail and will serve as the enrollment confirmation.

15.2 Randomization

On the completion of the second stage of registration, the CRDB automatically randomizes the participant to sulindac or placebo.

For AIMS and RCC:

The MSKCC RSA sends an email message to the randomization coordinator at MSKCC informing her that the second-stage of registration is complete. The participant's name, study ID and "CRDB MRN" are provided to the randomization coordinator who accesses the CRDB to identify the treatment assignment for the new participant. The randomization coordinator sends a *secure* email message directly to the pharmacist at AIMS or RCC. The message contains the participant's name, study ID, "CRDB MRN" and treatment assignment. The AIMS or RCC pharmacist sends a reply email message to the randomization coordinator to confirm the treatment assignment. The email messages are archived and a paper trail is maintained.

For MSKCC:
The MSKCC pharmacist accesses the CRDB to identify the treatment assignment for the new participant by matching the "MSKCC MRN" on the prescription, with the MRN stored in the CRDB. Drug is dispensed by the MSKCC pharmacist according to the Pharmacy.

16.1 DATA MANAGEMENT ISSUES

MSKCC will be the coordinating center for this trial, and will thus be responsible for all aspects of data management. Our study team, in collaboration with the Office of Clinical Research and the Office of the Physician-in-Chief, has spent a considerable amount of time and effort in developing a comprehensive data management plan (see Appendix VI. Data and Safety Monitoring Plan).

At least one Research Study Assistant (RSA) will be assigned to the study at each site. The responsibilities of the RSA includes project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol study team. All data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

16.1.1 Data and Source Documentation for Participating Sites

Data

Standardized Case Report Forms (CRFs), directions for use and sign off requirements have been generated for this study. Blank case report forms will be sent to the study staff at each participating site for use. The participating Site PI is responsible for ensuring these forms are completed accurately, legibly and in a timely manner.

Source Documentation

Source documentation refers to original records of observations, clinical findings and evaluations that are subsequently recorded as data. Source documentation should be consistent with data entered into CRFs. Relevant source documentation to be submitted throughout the study includes:

- Baseline measures to assess pre–protocol disease status (eg. Physical exam, oral cavity exam and lesion measurements, histology of lesion biopsy, blood chemistry and hematology)
- Treatment records
- Grade 3-5 toxicities/adverse events not previously submitted with SAE Reports
- Response designation

16.1.2 Data and Source Documentation Submission for Participating Sites

Participating sites should fax or e-mail CRFs and source documentation to MSKCC to the contact provided below. Submissions should include a cover page listing all CRFs enclosed per participant.

FAX: *212-557-0495* to the attention of *04-099* Research Staff

OR

EMAIL: Lasha Clarke clarkel@mskcc.org

MAILING ADDRESS:
Lasha Clarke
633 3rd Ave, Floor 15
New York, NY 10017

Participating sites are required to keep copies of each form that is sent via postal mail.

16.1.3 Data and Source Documentation Submission Timelines for Participating Sites

Data and source documentation to support data should be transmitted to MSKCC according to chart below:

	Baseline	*Weeks 4, 8, 12, 16, and 20*	*Adverse Event*	*Week 24 end of study/ Off Study*
SUBMISSION SCHEDULE				
Source Documentation	Within 24 hours (see section 15.1.1)	within 14 days of visit	Within 3 days of event (see section 17.2.1); updates to be submitted as available	Within 14 days of visit
CRFs	Within 7 days of visit			
Required Forms				
Subject Enrollment Form	X			X
Subject Randomization Form		X (*after baseline visit two only)		
Medical History Form	X			
Physical Exam	X	X		X
Clinical Laboratory Form	X			X
Concomitant Medications Form	X	X		X
Lesion/EOD Form		X		X
Adverse Event Form		X	X	X
Serious Adverse Event Form			X	
Off Study Form				X
Tobacco History	X			
Alcohol History	X			
Follow-up Tobacco Use Questionnaire		X		X
Tobacco Cessation Counseling		X		X
Compliance		X		X
Monthly Follow-Up Phone Calls		X(*weeks 2, 6, 10, 14, 18, and 22 ONLY)		
COX-2 by Quantitative PCR	X			
DNA Ploidy Analysis				X
Ki-67 Expression by Immunohistochemistry				X
p53 Expression by Immunohistochemistry				X
Verification Form				X

16.1.4 Data Review and Queries for Participating Site Data

Research staff at MSKCC will review data and source documentation as it is submitted. Data will be monitored against source documentation and discrepancies will be sent as queries to the participating

sites. Queries will be sent by MSKCC Research staff twice a month.

Participating sites should respond to data queries within 14 days of receipt.

16.1 Quality Assurance

16.1.1 Quality Assurance for Participating Sites

Each site participating in the accrual of participants to this protocol will be audited by the staff of the MSKCC study team for protocol and regulatory compliance, data verification and source documentation. Audits may be accomplished in one of two ways: (1) selected participant records can be audited on-site at participating sites or (2) source documents for selected participants will be sent to MSKCC for audit. Audits will usually be determined by participant accrual numbers and rate of accrual, but can also be prompted by reported SAEs or request of MSKCC PI.

Audits will be conducted at least once shortly after initiation of participant recruitment at a site, annually during the study (or more frequently if indicated), and at the end or closeout of the trial. The number of participants audited will be determined by available time and the complexity of the protocol.

The audit will include a review of source documentation to evaluate compliance for:

- Informed consent documents and procedures
- Adherence to eligibility criteria
- Protocol defined treatment
- Required baseline, on study and follow-up protocol testing
- IRB documents (submitted amendments, annual continuing review reports, SAEs)
- Required specimen submission
- Case Report Form submissions to MSKCC: timelines and accuracy

A wrap-up session will be conducted at the participating site and preliminary findings will be discussed with the participating site PI and research team. The preliminary results will be sent to the MSKCC PI.

Each audit will be summarized and a final report will be sent to the PI at the audited participating site within 30 days of the audit. The report will include a summary of findings, participant by participant case review, specific recommendations on any performance and/or shortcomings and request for corrective action, when necessary. When corrective action is required, the participating site must reply within 45 days of receipt of audit report with their corrective action plan.

A copy of the audit report and corrective action plan (if applicable) submitted by the participating site must be sent to the MSKCC IRB/PB, CRQA and maintained in the department's protocol regulatory binder.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute

for Data and Safety Monitoring of Clinical Trials" which can be found at: http://deainfo.nci.nih.gov/grantspolicies/datasafety.htm. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb5.mskcc.org/intranet/_assets/_tables/content/359709/DSMPlans07.pdf*

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

In addition, our study team in collaboration with the Office of Clinical Research and the Office of the Physician-in-Chief has spent a considerable amount of time and effort in developing a tailored comprehensive data and safety management plan specific to our pilot multi-center international clinical chemoprevention trial. Please refer to see Appendix VI to view our detailed and rigorous data and safety monitoring plan.

16.3 Regulatory Documentation

Prior to implementing this protocol at MSKCC, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MSKCC Institutional Review Board/Privacy Board (IRB/PB). Prior to implementing this protocol at the participating sites, approval for the MSKCC IRB/PB approved protocol must be obtained from the participating site's IRB.
The following documents must be provided to MSKCC before the participating site can be initiated and begin enrolling participants:

- Participating Site IRB approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Participating Site IRB approved consent form
- Participating Site IRB membership list
- Participating Site IRB's Federal Wide Assurance number and OHRP Registration number
- Curriculum vitae and medical license for each investigator and consenting professional
- Documentation of Human Subject Research Certification training for investigators and key staff members at the Participating Site
- Participating site laboratory certifications and normals

Upon receipt of the required documents, MSKCC will formally contact the site and grant permission to proceed with enrollment.

16.3.1 Amendments

Each change to the protocol document must be organized and documented by MSKCC and first approved by the MSKCC IRB/PB. Upon receipt of MSKCC IRB/PB approval, MSKCC will immediately distribute amendments to the participating sites, for submission to their local IRBs.

Participating sites must obtain approval for all non expedited amendments from their IRB within 90 calendar days of MSKCC IRB/PB approval. If the amendment is the result of a safety issue or makes eligibility criteria more restrictive, sites will not be permitted to continuing enrolling new participants until the participating site IRB approval has been granted.

The following documents must be provided to MSKCC for each amendment within the stated timelines:

- Participating Site IRB approval
- Participating Site IRB approved informed consent form and HIPAA authorization

16.3.2 Additional IRB Correspondence

Continuing Review Approval

The Continuing Review Approval letter from the participating site's IRB and the most current approved version of the informed consent form should be submitted to MSKCC within 7 days of expiration. Failure to submit the re-approval in the stated timeline will result in suspension of study activities.

Deviations and Violations

A protocol deviation on this study is defined as a request to treat a research participant who does not meet all the eligibility criteria, pretreatment evaluation, or who requires alteration in their study plan. If a deviation from this protocol is proposed for a potential or existing participant at MSKCC or a participating site, approval from the MSKCC IRB/PB is required prior to the action. Participating sites should contact the MSKCC PI who will in turn seek approval from the MSKCC IRB/PB.

A protocol violation is anything that occurs with a participant, which deviated from the protocol without prior approval from the MSKCC IRB/PB. For protocol violations that are identified after they occur, the participating site should report to MSKCC as soon as possible. The MSKCC PI will in turn report the violation to the MSKCC IRB/PB.

Participating sites should report deviations and violations to their institution's IRBs as soon as possible per that site's institutional guidelines. Approvals/acknowledgments from the participating site IRB for protocol deviations and violations should be submitted to MSKCC as received.

Other correspondence

Participating sites should submit other correspondence to their institution's IRB according to local guidelines, and submit copies of that correspondence to MSKCC.

16.3.3 Document maintenance

The MSKCC PI and the Participating Site PI will maintain adequate and accurate records to enable the implementation of the protocol to be fully documented and the data to be subsequently verified.

The participating sites will ensure that all participating site IRB correspondence (IRB approval letters referencing protocol version date and amendment number, IRB approved protocol, appendices, informed consent forms, deviations, violations, and approval of continuing reviews) is maintained in the regulatory binder on site and sent to MSKCC.

A regulatory binder for each site will also be maintained at MSKCC; this binder may be paper or electronic.

After study closure, the participating site will maintain all source documents, study related documents and CRFs for 3 years.

16.4 Noncompliance
If a participating site is noncompliant with the protocol document, accrual privileges may be suspended and/or contract payments maybe withheld (if applicable), until the outstanding issues have been resolved.

17.1 PROTECTION OF HUMAN SUBJECTS

Human subjects will be provided with complete informed consent and all pertinent questions answered. These encounters will be documented in the medical record. Standard of care treatment will be provided for all subjects. The suspicion of disease progression of disease will trigger biopsy of lesions and withdrawal from the study. Incremental costs of research are not charged to subjects or their insurance carriers. Every effort is made to preserve the subjects' rights during the recruitment and conduct of the study.

Inclusion of Children in Research

This protocol/project does not include children because the number of children is limited and because the majority are already accessed by a nationwide pediatric cancer research network. This statement is based on exclusion 4b of the NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects.

17.2 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board.

17.3 Serious Adverse Event (SAE) Reporting

Our study team in collaboration with the Office of Clinical Research and the Office of the Physician-in-Chief has spent a considerable amount of time and effort in developing a tailored serious adverse event reporting plan specific to our pilot multi-center international clinical chemoprevention trial (Appendix VI).

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org containing the following information:

Fields populated from the CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following information:
  - A explanation of how the AE was handled
  - A description of the subject's condition
  - Indication if the subject remains on the study
  - If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.

17.3 Serious Adverse Event (SAE) Reporting for Participating Sites

Responsibility of Participating Sites

- Participating sites are responsible for reporting all SAEs to the MSKCC PI via fax or e-mail within 3 calendar days of learning of the event.
- Participating sites should notify the MSKCC PI of any grade 5 event immediately.
- Participating sites should use the SAE Report Template (appendix IX) to report SAEs to MSKCC.

SAE contact information for the Coordinating Center is listed below:

Principal Investigator:Jay O. Boyle, MD, 212-639-2906

Research Staff: Lasha Clarke, 646-227-2224

Responsibility of MSKCC

- The MSKCC Research Staff is responsible for submitting all SAEs to the MSKCC IRB/PB as specified in 17.2
- The MSKCC PI is responsible for informing all participating sites about unexpected SAEs within 30 days of receiving the stamped SAE from the MSKCC IRB/PB.
- Any report pertaining to a grade 5 event will be distributed to the participating sites as soon as possible.

17.4 Safety Reports

- MSKCC will distribute outside safety reports to the participating sites immediately upon receipt.
- MSKCC must submit safety reports to the MSKCC IRB/PB according to institutional guidelines.
- Participating sites must submit safety reports to their institution's IRBs within 30 days of receipt from MSKCC or per participating site guidelines.

18.1 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

The investigators listed on the protocol cover page and their qualified designees at each participating site may obtain informed consent and care for the participants according to good clinical practice and protocol guidelines.

Signed copies of the informed consent should be distributed as follows: One copy will be given to the participant to be retained for their personal records. One copy will be maintained on file at the MSKCC. The third copy will be confidentially maintained by the participating institution.

A note will be placed in the medical record documenting that informed consent was obtained for this study, and that the participant acknowledges the risk of participation.

For AIMS and RCC subjects: three original informed consent forms will be obtained: 1) one consent form will be kept secure in the office of Dr. Kuriakose or Dr. Sebastian in the subject's research and/or medical record, 2) one of the originals will be sent to MSKCC in batches as appropriate (see Appendix VI, Section D.2.b). A copy of the signed consent will be faxed to the RSA at MSKCC for registration onto the protocol, inclusion in the research record and entry into the CRDB at the time of consent, 3) one signed informed consent will be provided to the subject.

For MSKCC subjects: three original informed consent/RA will be obtained: 1) one consent/RA form will be kept secure in the research office of the RSA, 2) another consent/RA form will be used in the electronic medical record and 3) one signed informed consent/RA will be provided to the subject.

18.1 For Participating Sites

The investigators listed on the protocol cover page and their qualified designees at each participating site may obtain informed consent and care for the participants according to good clinical practice and protocol guidelines.

Signed copies of the informed consent should be distributed as follows: One copy will be given to the participant to be retained for their personal records. One copy will be maintained on file at the MSKCC. The third copy will be confidentially maintained by the participating institution.

A note will be placed in the medical record documenting that informed consent was obtained for this study, and that the participant acknowledges the risk of participation.

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20.1 APPENDICES

I. Informed Consent Form.

II. Eligibility Form.

III. Case Report Forms.

IV. Pill Diary and Adverse Event Form.

V. Shipping Instructions.

VI. Data and Safety Monitoring Plan

VII. Description of Amrita Institute for Medical Sciences and Research Centre (AIMS).

VIII. Description of Regional Cancer Centre (RCC).

IX. Zubrod Performance Status

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